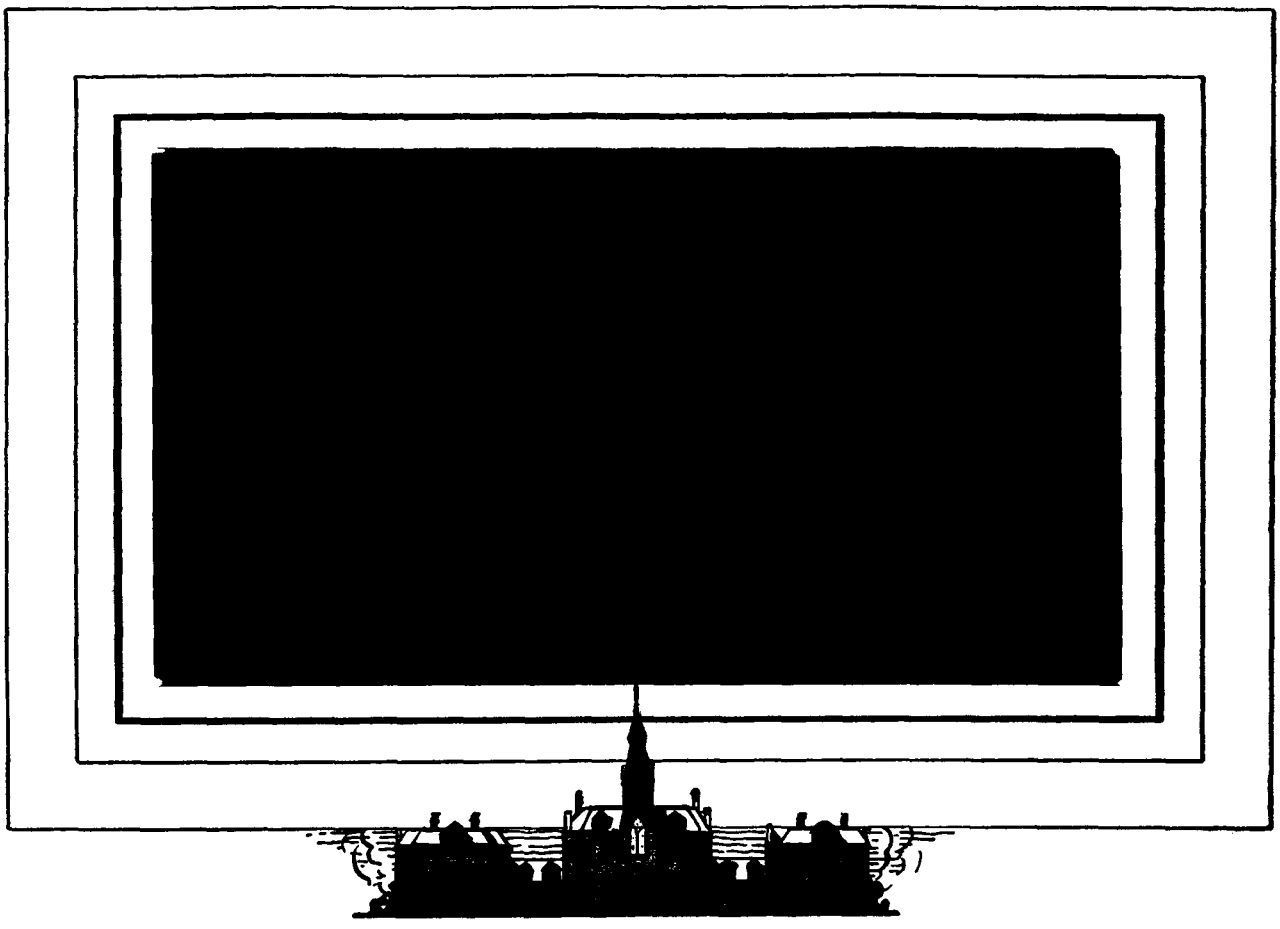

KANSAS STATE UNIVERSITY

MANHATTAN, KANSAS

STATUS REPORT

October 6, 1964 to April 6, 1965

NASA Grant NsG-292-63/17-001-001

Submitted by
W. S. Ruliffson
Principal Investigator
Department of Biochemistry
Willard Hall, Room 32
Kansas State University
Manhattan, Kansas

Ethylene production by plants has been recognized for some time (1,2,3,4,5,6). Not so well known or recognized, however, is the evolution of ethylene by a variety of mammalian cell systems or preparations. Thus Chandra and Spencer (7) have demonstrated ethylene evolution from aged and sonicated rat liver and intestinal fractions. Gibson (8) has demonstrated ethylene production by a beef heart mitochondrial suspension, incubated at 30°C in a pyruvate-malate medium, at the rate of 40.7 mul of C_2H_4/2 hr. from 46 ml of a 51 mg. protein/ml suspension. These mitochondria consumed oxygen and carried out oxidative phosphorylation. Ethylene production from sonicated mitochondria increased 10 fold. The fractionation of sonicated mitochondria left ethylene-producing activity almost entirely in the supernatant.

Certain biochemical effects of ethylene on isolated mitochondria have been described by Lyons and Pratt (9) who showed, by using spectrophotometric techniques for following the changes produced, that ethylene, in an overlaying ethylene-air mixture, caused swelling of mitochondria suspended in 0.125M KCl. Ethylene in a concentration of 1000ppm did not enhance oxygen uptake or uncouple oxidative phosphorylation. These authors suggest that ethylene causes increased mitochondrial membrane permeability.

---

1. Spencer, M., Nature, 184, 1231, (1959)
2. Burgis, P., Ann. Rev. Plant Phys., 13, 265, (1962)
3. Burg, S. P., and Burg, E., Nature 203, 869, (1964)
4. Lieberman, M., et al, Nature 204, 756, (1964)
5. Meherimk, M., and Spencer., M., Nature 204, 43, (1964)
6. Spencer M., and Olson, A. O., Nature 205, 699, (1965)
7. Chandra, G. R. and Spencer, M., Nature 197, 366, (1963)
8. Gibson, M., Biochim. et Biophys. Acta, 78, 528, (1963)
9. Lyons, J. M. and Pratt, H. K., Arch. Biochem. and Biophys. 104, 318, (1964)

Lieberman and Mapson (10) have demonstrated non-enzymatic evolution of certain hydrocarbons by exposing linolenic acid to air, light and 20-25°C for a minimum of three days and then incubating with ascorbic acid and copper ion in acetate buffer, pH 4.5. Ethylene and ethane were produced in a ratio of 11:15.

These findings may be summarized as follows: 1) Small amounts (2.3×10^{-12} moles/gm wet weight) of ethylene have been shown to be evolved by liver, heart, and intestinal mitochondria 2) In plants at least, Kreb's cycle intermediate contribution to ethylene production appears to be negligible; 3) ethylene appears to effect mitochondrial swelling, but has not yet been shown to affect oxygen consumption or oxidative phosphorylation; 4) the most likely site for ethylene production may be certain mitochondrial lipides.

Ethylene production by other than plant cells, especially by a variety of mammalian cells, is a rather unexpected finding. This immediately poses the prospect that a variety of other unexpected (foreign?) volatile metabolites generated by preparations of normal cells, might be shown, provided the means for detection and identification of volatile materials appearing in extremely dilute concentrations were available.

Our approach to this problem may be briefly described as follows. The intact liver, excised from a laboratory rat sacrificed by stunning was homogenized in phosphate-glucose-Ringer's solution using the conventional Potter-Elvejhm apparatus. The final 10% homogenate was incubated at room temperature (23°C) for four hours. The "dead" space gas overlaying the homogenate, was sampled by introducing aliquots into a pre-evacuated (10^{-4} mm Hg) ambient temperature gas inlet system. This aliquot was in turn, bled into the source of a model 14-101 Bendix Time-of-Flight Mass Spectrometer. Sample pressures were of the order of 100-1000 u Hg; instrument

---

10. Lieberman M. and Mapson, K. A. Nature, 204, 243, (1964)

pressures of the order of 2-10 X 10^{-7} mm Hg. Location and intensity of mass number peaks were monitored with a model 543A Tektronix oscilloscope and recorded by means of a Honeywell oscillograph. Sample inlet system and mass spectrometer background contributions were recorded before and after sample introduction. Filament current was 2.7 amps, trap current 0.15 u amps, analog (#2) sensitivity 0.1 X 1, scan rate 5, total current 0.1 mu amps, oscillograph preamplifier set at full gain.

Experiment 1 consisted of the sample preparation and conditions described above. In experiment II, sample preparation was altered to include the addition of several antibiotics to obviate any possible bacterial volatile compound contribution. Also, excess O_2, used for homogenate oxygenation, was evacuated by means of water line aspirator, thus accounting for the disappearance of mass 32 in experiment II. Mass numbers listed are those remaining following elimination of background contributions. Raw data from which these mass numbers were taken is tabulated in Appendix I.

M/e Experiment I	M/e Experiment II	Similar Mass Numbers
16, 28, 32, 33,	30, 43, 44, 57,	43, 44, 57, 58,
40, 42, 43, 44,	58, 59, 60, 67,	59, 60, 71, 73
46, 57, 58, 59,	71, 72, 73, 74,	74, 75, 89?,
60, 63, 71, 73,	75, 76, 77, 78,	108?
74, 75, 89, 96,	79, 83, 84, 85,	
108	89, 90, 108,	
	138-142	

Tentative Mass Number Assignments

43 $H_3C-\overset{O}{\overset{\|}{C}}{}^{+}$; $H_3C-CH_2-CH_2^+$

44 CO_2^+; $H_3C-\overset{O}{\overset{\|}{C}}-H^+$

57 $H_3C-\underset{O}{\overset{O}{\overset{\|}{C}}}-CH_2^+$; $H_3C-CH_2-CH^+-CH_3$

58 $H_3C-\underset{O}{\overset{O}{\overset{\|}{C}}}-CH_3^+$

59 $H_3C-CH_2-CH_2-NH_2^+$

60 $H_3C-CH_2-CH_2-OH^+$

71 $H_3C-\underset{O}{\overset{O}{\overset{\|}{C}}}-CH_2-CH_2^+$; $H_3-C\overset{O}{\overset{\|}{C}}-CH_2-CH_2^+$

73 $H_3C-CH_2-CH_2-CH_2O^+$; $H_3C-CH(OH)-\overset{O}{\overset{\|}{C}}{}^{+}$; $H_3C-CH_2-CH_2-CH_2-NH_2^+$

74 $H_3C-CH_2-CH_2-CH_2-OH^+$; $H_3C-CH(OH)-\overset{O}{\overset{\|}{C}}-H^+$

75 $H_3C-CH(OH)-CH(OH)^+$?; $H_3C-CH(OH)-CH_2-NH_2^+$

89 ?

108 ?

One may note that mass number 28 appears (23% of base peak, mass no. 32) in experiment I, but not Experiment II. The appearance of mass 28 is usually due to N_2^{+1} resulting from ionization of leak-introduced air. Obviously, air would also give rise to mass number 32 (O_2^{+1}). In this case, however, base peak 32 is due to the use of oxygen for enhancement of homogenate respiration. As a consequence, all air was driven from the sample bulb and air-borne nitrogen virtually eliminated.

In addition, electron energy levels used were such that ethylene, if present, but not N_2, would undergo molecular ion formation. In our opinion then, mass number 28 in Experiment I could be due to the ethylene molecular ion ($H_2C{=}CH_2^+$). Why this same mass number failed to appear in experiment II remains to be determined.

It would appear that a natural extension of the techniques and findings herein described would consist of the following:

1) Further deliniation of present work to more clearly ascertain significant mass number contribution 1) particularly at low electron energy levels (molecular ion formation). 2) with the elimination of species known to be present, i.e., $N_2^{+2} = 14$, $O_2^{+2} = 16$, $H_2O^+ = 18$, $N_2^+ = 28$, $O_2^+ = 32$, $CO_2^+ = 44$, and 3) from mass 60 up through 138-142.

2) Detectable differences in mass number contribution when mitochondrial, microsomal, supernatant, or subfractions of these, prepared by sonication, or other acceptable procedures, are employed, rather than the more complex, from a systems point of view, homogenate.

3) Typical mass number patterns discerned when homogenates, or the various subfractions, prepared from microbial cells are similarly examined.

4) The effect on mass number patterns when certain mitochondrial swelling agents, e.g., ATP, Ca^{+2}, throxine, genistein, or antimetabolites, e.g., malonate, dinitrophenol, triamcinalone, are added to mitochondrial, or other appropriate preparations.

Certain other modifications, either in volatile compound sample preparation, or in additional instrumentation may provide the means for further refinement and evaluation of amounts and types of volatile materials evolved by these preparations. I refer primarily to utilizing the head-space gas technique for sample preparation, and the use of the gas chromatograph as a means of individual compound identification and introduction into the mass spectrometer, once we have a better idea of the minimum sample volumes from which we can reasonably expect to demonstrate volatile compound evolution.

APPENDIX

EXPERIMENT I

1	2	3	4	5
M/e (a)	Intensity, % of base peak (b)	Electron Energy (V) (c)	Base peak and time interval (d)	Sample Pressure (u Hg) (e)
16	22	12.5	32/60	1000
28	23	12.5	32/60	1000
32	100	12.5	32/60	1000
33	70	12.5	32/60	1000
40	24	12.5	42/0	800
42	100	12.5	42/0	800
42	100	12.5	42/0	500
42	47	12.5	126-130/120	200
43	100	12.5	43/0	300
44	19	12.5	58/0	400
44	18	12.5	126-130/120	200
46	17	14	63/30	100
57	10	12.5	42/0	800
57	78	14	63/30	100
57	41	12.5	73/60	500
57	18	12.5	126-130/120	200
58	100	12.5	58/0	500
58	86	12.5	43/0 + 60/0	300
58	100	12.5	58/0	400
58	44	14	63/30	100
58	41	12.5	108/60	500
59	78	14	63/30	100
59	87	12.5	108/60	500
59	50	12.5	73/60	500
60	85	12.5	42/0	800
60	100	12.5	60/0	300
60	70	12.5	58/0	400
60	48	12.5	58/0	1000
63	100	14	63/30	100

(a) Mass to charge ratio. Thus O^{+1}=32/1=32; O_2^{+2}=32/2=16; H_2O^{+1}=18/1=18; Hg^{+1}=198-202/1=198, 199, 200, 201, 202; Hg^{+2}=198-202/2=99, 99.5, 100, 100.5, 101

(b) Base peak (column 4) interpreted as being that component giving maximum oscillograph galvanometer deflection under the described sensitivity and other conditions. All other peak heights appearing on the appropriate oscillograph trace are the stated part (or percentage) of the base peak.

(c) These values are direct readings from instrument panel dial used to adjust voltage passing through filament electrodes.

(d) These figures indicate the time (minutes) after incubation period, at which the base peaks indicated, were isolated in the gas inlet system from the dead space gas overlaying the homogenate.

(e) Phillips gauge reading of sample pressure in ambient temperature gas inlet system.

Table I (cont)

M/e	Intensity, % of base peak	Electron Energy (V)	Base peak and time interval	Sample Pressure (u Hg)
71	36	14	126-130/120	200
73	18	14	108/60	^{o}500
73	100	14	73/60	$^{\Delta}$500
74	86	14	73/60	500
75	35	14	43/0 & 60/0	300
75	9	14	108/60	^{o}500
75	58	14	73/60	$^{\Delta}$500
75	19	14	126-130/120	200
89	63	14	108/60	500
89	58	14	73/60	$^{\Delta}$500
96	42	14	73/60	$^{\Delta}$500
108	100	14	108/60	$^{\Delta}$500

EXPERIMENT II

M/e	% Intensity, of Base Peak	Electron Energy (V)	Base peak per time *min	Sample Pressure (u Hg)
30	15	12.5	75/0	1000
43	58	12.5	60/30 & 75/30	500
43	43	12.5	57,59,60,75/30	400
43	19	11.0	75,76,108/60	700
43	78	11.0	138-142/60	25
44	16	11.0	75/60	800
57	100	12.5	57,59,60,75/30	400
57	100	12.5	75,78,57/0	200
58	58	12.5	75,60/0	800
59	40	12.5	75/0	1000
59	40	12.5	75 + 60/0	800
59	34	12.5	75,78,57/0	200
59	47	12.5	60 + 75/30	500
59	100	12.5	57,59,60,75/30	400
59	100	11.0	59/60	1000
59	45	11.0	75/60	800
60	100	12.5	60 + 75/30	500
60	79	12.5	75/0	1000
60	100	12.5	75 + 60/0	800
60	58	11.0	75,76,108/60	700
60	100	12.5	57,59,60,75	400

^{o} 4 X 10^{-7} B. A. Gauge reading
$^{\Delta}$ 6 X 10^{-7} B. A. Gauge reading
* Zero time corresponds to 2 hrs. incubation time

M/e	%Intensity, of base peak	Ev	Base peak per time *min	Sample Pressure (u Hg)
60	55	11.0	75/60	800
67	33	12.5	57,59,60,75/30	400
71	24	12.5	75/0	1000
71	65	11.0	75,76,108/60	700
72	58	12.5	75,60/0	800
73	58	12.5	75,78,57/0	200
74	40	12.5	60,75/30	500
75	100	12.5	75/0	1000
75	100	12.5	75,60/0	800
75	100	12.5	75,78,57/0	200
75	100	12.5	60,75/30	500
75	100	12.5	57,59,60,75/30	400
75	15	11.0	108/30	50
75	30	11.0	59/60	1000
75	100	11.0	75/60	800
75	100	11.0	75,76,108/60	700
75	100	11.0	75/60	500
76	33	12.5	75,60/0	800
76	47	12.5	60,75/30	500
76	45	12.5	57,59,60,75/30	400
76	100	11.0	75,76, 108/60	700
76	10	11.0	75/60	500
77	9	11.0	75/60	500
78	100	12.5	57,75,78/0	200
79	27	11.0	75/60	800
83	79	11.0	75/60	500
84	8	12.5	75,78,57/0	200
84	10	11.0	75/60	500
85	58	12.5	75,60/0	800
89	76	12.5	75/0	1000
89	12	11.0	75,76,108/60	700
90	79	12.5	75/0	1000
108	100	11.0	75,76,108/60	700
108	64	11.0	75/60	500
138-142	100	11.0	138-142/160	25